

AD \_\_\_\_\_

AWARD NUMBER DAMD17-96-1-6169

TITLE: Estrogen Receptor-Mediated Transcription In Vitro

PRINCIPAL INVESTIGATOR: Hong Liu, M.D., Ph.D.

CONTRACTING ORGANIZATION: Northwestern University  
Evanston, Illinois 60208

REPORT DATE: December 1998

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;  
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20001130 020

DTIC QUALITY INSPECTED 4

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE December 1998		3. REPORT TYPE AND DATES COVERED Annual (1 Dec 97 - 30 Nov 98) Summary
4. TITLE AND SUBTITLE Estrogen Receptor-Mediated Transcription In Vitro			5. FUNDING NUMBERS DAMD17-96-1-6169	
6. AUTHOR(S) Hong Liu, M.D., Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Northwestern University Evanston, Illinois 60208			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words)				
14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 16	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

## FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

\_\_\_\_ Where copyrighted material is quoted, permission has been obtained to use such material.

\_\_\_\_ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

\_\_\_\_ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

\_\_\_\_ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

✓ \_\_\_\_ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

\_\_\_\_ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

\_\_\_\_ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

\_\_\_\_ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

H. Liu 2/99

PI - Signature

Date

## TABLE OF CONTENTS

Cover

SF 298 (Report Documentation Page)

Foreword

Table of Contents

Summary	2
Introduction	2
Experimental Procedures	3
Results	4
Conclusions	9
References	10

## SUMMARY

Estrogen receptor (ER) is a ligand-activated transcription activator. To elucidate the mechanism of ER-mediated transcription in detail, we studied transcriptional activity of the ER *in vitro*. We demonstrated ER-mediated transcription in a cell-free transcription system is ligand-dependent. Antiestrogens ICI164,384, ICI182,780 and 4-hydroxytamoxifen significantly inhibited transcriptional activity of the ER. Estradiol overcame the inhibitory effect of the antiestrogens and induced ER-mediated transcription. Under the condition used for transcription assays, ICI164,384 and ICI182,780 inhibited ER-ERE complex formation which might contribute to the inhibitory effect of these antiestrogens on ER-mediated transcription. 4-hydroxytamoxifen changed the mobility of ER-ERE complex in the gel mobility shift assay, suggesting a conformational change of the complex. Steroid receptor coactivator 1 (SRC-1) significantly augmented ER-mediated-transcription *in vitro*. The hormone binding domain (HBD) of the ER that binds to estrogen receptor associated proteins (ERAPs) in a ligand-dependent manner inhibited ER-mediated transcription *in vitro*. In contrast, the truncated ER HBD (ER $\Delta$ 534) lacking of 535 to 595 amino acids of the ER which binds to estradiol but does not associate with ERAPs did not affect ER-mediated effect in this study, suggesting that ERAPs are required for the full transactivation activity of the ER.

## INTRODUCTION

Estrogen receptor (ER) is a member of nuclear hormone receptor superfamily which includes steroid hormone receptors, thyroid and retinoid hormone receptors, vitamin D receptor, and a large number of so-called orphan receptors for which no ligands have been identified (1-4). These receptors function as ligand-activated transcription factors. The ER was identified in 1960s and the function of the ER as a transcription regulator was also proposed (5, 6). Since then, extensive studies have been conducted to probe the detail mechanism of ER-mediated effects at molecular levels. With the cloning of the ER gene (7, 8), significant progresses have been made in the elucidation of the structure of the ER and the dissection of the mechanism of ER-mediated signal transduction. Human ER is a protein of 595 amino acids and has a molecular weight of approximately 67 kDa (7, 8). Like all members of the nuclear hormone receptor superfamily, it has A to F domains from N-terminus to C-terminus and has a structure which includes a C-terminal domain with hormone binding, dimerization and hormone-dependent activation function 2 (AF2), a hinge region, a highly conserved central DNA binding domain (DBD) with two zinc fingers, and an N-terminal domain which has autonomous transcription activation activity (AF1) in a cell- and promoter-specific manner. Although different mechanisms have been proposed, most studies on ER-mediated signal transduction have been carried out on the classic ER-ERE pathway in which the ER binds to estrogen, forms a

homodimer, recognizes and binds to a palindromic cognate estrogen receptor responsive elements (EREs) with a consensus sequence of GGTCANNNTGACC which locates in the regulatory regions of ER targeted genes and then regulates gene transcription (9-14).

However, the detailed mechanism of ER-mediated transcription is still unknown. There is evidence that ER facilitates the formation of the initiation complex (15). The extensive studies on ER-mediated signal transduction led to the discovery of a set of ER associated proteins (ERAPs) which gives a much more sophisticated view of the mechanism of nuclear receptor action. Up to now, several nuclear receptor associated proteins have been identified or cloned in different laboratories, including ERAP140 (16), RIP140 (17), SRC-1 and related proteins (18-24), p300/CBP (25-28), SWI2/SNF2 (29), TIF1 (30), and TRIP1 (31). These nuclear receptor associated proteins bound to nuclear receptors in ligand-dependent manners correlates to the ligand-dependent transcription of the receptors in cells, suggesting a putative role of the nuclear receptor associated proteins in ligand-activated receptor-mediated transcription.

In this study, a cell-free transcription system was used to study the effects of ER-associated proteins on ER-mediated transcription. We showed that ER-mediated transcription *in vitro* was ligand-dependent under the condition used in this study. ER hormone binding domain associated proteins were required for the full transcription activity of ER. GST-SRC-1 enhanced ER-mediated transcription *in vitro*.

## EXPERIMENTAL PROCEDURES

**Plasmids** — pAdMLPERE, pAdMLPERE2 and pAdMLPERE3 are described previously (32) (kindly provided by Dr. C. Abbondanza), which contains three copies of ERE linked to a minimal adenovirus major late promoter (-53 to +9) and a 400 nucleotides of G-less cassette as the reporter. The internal control (pAdMLPmERE) which has the same promoter and a shorter G-less cassette (200 nucleotides) was generated by replacing the three copies of ERE by a mutated ERE (5' -AGGACACAGTGTCT- 3') which abolishes the formation of ER-ERE complex (33). GST-HBD3, GST-Δ534 are described previously (16). hSRC-1 cDNA that is the equivalent of that reported by Onate et al (18) was subcloned as GST-SRC-1 and expressed in *E. Coli* strain Y1090 strain.

**Protein Expression**—Human ER is overexpressed in baculovirus system and partially purified by Mono S column (PanVera Inc.). GST-HBD3, GST-Δ534 and GST-SRC-1 are expressed in bacteria and purified on glutathione sepharose (Pharmacia).

**In Vitro Transcription Assay**—HeLa cells were grown in suspension and nuclear extract was prepared as previously described (34). The *in vitro* transcription reaction contained: 10 mM HEPES (pH 7.9), 8.5% glycerol, 60 mM KCl, 7.5 mM MgSO<sub>4</sub>, 5 mM creatine phosphate, 2.5 mM DTT, 30 U RNasin inhibitor, 5 mg/ml BSA, 12.5 mM ATP and UTP, 5 μM CTP, 40 μM 3-

O'-methy-GTP, 20  $\mu$ Ci [ $\alpha$ - $P^{32}$ ]CTP (800 Ci/mmol), 10 U RNase T1. Different concentration of templates (pAdMLPERE3 and pAdMLPmERE) were used. The ER was incubated with hormones at 30°C for 20 minutes, then incubated with HeLa nuclear extract (30 - 40  $\mu$ g) at 30°C for another 20 minutes followed by 30 minute incubation with templates at 30°C. The transcription was initiated by adding NTPs. Reactions were stopped by addition of 200  $\mu$ l of stopping buffer (10 mM Tris (pH 7.9), 10 mM EDTA, 1 M ammonium acetate, 0.5% SDS, and 70  $\mu$ g/ml yeast tRNA). The RNA was extracted once with phenol/chloroform/isoamyl alcohol (25/24/1) and once with chloroform/isoamyl alcohol (24/1), precipitated with 100% ethanol and separated on a 6% sequencing gel. The gel was autoradiographed at -70°C with intensifying screens.

**Gel Shift Assay**—ERE (5'-GATCTCTTTGATCAGGTCACCTGTGACCTGACTTTG-3') oligonucleotides were annealed and labeled with [ $\alpha$ - $P^{32}$ ]dGTP (3000 Ci/mmol) by Klenow fragment. The ER was incubated under the same condition as that for *in vitro* transcription assay except that  $P^{32}$ -ERE was used instead of plasmid templates. The ER-ERE complex was separated on a 4% polyacrylamide gel in 0.5 X TGE (33).

**Preparation of ERAP-Depleted HeLa Nuclear Extract**—GST-HBD3 was Immobilized on GSH-sepharose (Pharmacia). HeLa nuclear extracts were incubated with the immobilized GST-HBD3-sepharose in the absence or presence of 10 nM  $E_2$  or 1  $\mu$ M 4-OHT. The supernatants were incubated with fresh GST-HBD3-sepharose. The procedure was repeated three times. The final supernatants were the depleted HeLa nuclear extracts (dNE). The ER associated protein levels were determined by Far-Western blot (16).

## RESULTS

**Baculovirus-Expressed Human ER Induces Transcription *in Vitro*** — To study the function of the ER *in vitro*, a cell free transcription system was set up as described previously (32, 35).

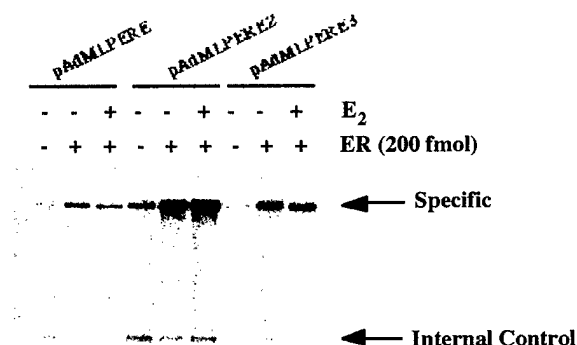


Figure 1. Stimulation of transcription *in vitro* by Partially purified baculovirus expressed human ER. The transcriptional activity of ER was analyzed by *in vitro* transcription assay as described under "Experimental Procedures". Two hundred fmol of ER and 1  $\mu$ M  $E_2$  and 10 ng of the templates were used. The specific transcript (upper arrow) is from ERE-containing templates pAdMLPERE, pAdMLPERE2 or pAdMLPERE3. The internal control transcript is from pAdMLPmERE template.

Figure 1 shows that the partial purified human ER expressed in baculovirus did not affect transcription on the internal control template which contains a mutated ERE site; it did significantly increase transcription from the specific template with one to three copies of ERE sites in the absence of estradiol ( $E_2$ ) (Fig. 1). However,  $E_2$  did not further increase ER-mediated transcription (Fig. 1) as reported previously (15, 32). Although the amount of HeLa nuclear extract (30 to 60  $\mu$ g) did not have significant effect on ER-mediated transcription *in vitro* (data not shown), the concentration of the specific template was a critical factor on ER-mediated effect in this system as shown in Figure 2.

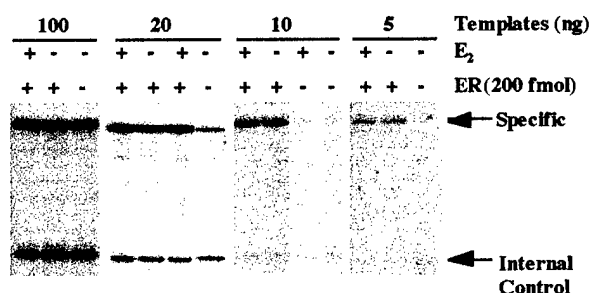


Figure 2. The concentration of the DNA template (pAdMLPERE3) had an important effect on ER-mediated transcription *in vitro*. The assay was performed as described in Figure 1 except that various amounts of templates (present in ng) were used.

When a high concentration of template such as 100 ng/reaction is used, the ER did not stimulate transcription from the ERE-containing template because of the high basal activity. However, the ER significantly increased the level of the specific transcript when 20 ng/reaction or less of the template is used.

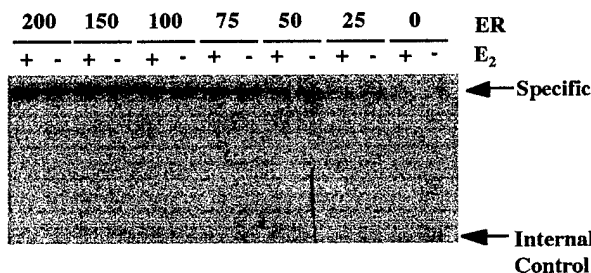


Figure 3. The concentration-dependent effect of the ER on ER-mediated transcription *in vitro*.

Figure 3 elucidated a concentration-dependent effect of the ER on ER-mediated transcription *in vitro*. With increasing concentration of the ER, ER-mediated transcription increased. However, the ER did not further stimulate transcription at the concentrations higher than 400 fmol (data not shown), and as the matter of fact the transcription level slightly decreases as shown previously (15).

*ER-Mediated Transcription in Vitro is Ligand-Dependent* — ER activated ERE containing

promoter in the absence of  $E_2$  *in vitro* which was different from the situation *in vivo*. There are two potential possibilities for the  $E_2$  independency. First, using circular plasmids as the templates *in vitro* instead of chromatin templates *in vitro* rules out the requirement of estrogen to activate ER function. This issue has been addressed in a recent publication. The other possibility is that there was estrogenic activity in the cell free transcription system. The endogenous estrogenic activity in the system might activate transcriptional activity of the ER in the absence of exogenous  $E_2$ . To study hormone-dependent effect on ER-mediated transcription *in vitro*, three antiestrogens including 4-hydroxytamoxifen (4-OH TAM), ICI164,384 and ICI182,780 were tested in the system. ICI164,384 and ICI182,780 are pure ER antagonists and 4-OH TAM is a partial ER agonist and antagonist (36, 37). In Figure 4, the ER stimulated transcription from the specific template independent of  $E_2$  as shown earlier. ICI164,384 significantly inhibits ER-mediated transcription in a concentration-dependent manner. When exogenous  $E_2$  was added,  $E_2$  overcomes the inhibitory effect of ICI164,384 and induces ER-mediated transcription. Similar results are obtained when ICI182,780 and 4-OH TAM were used.

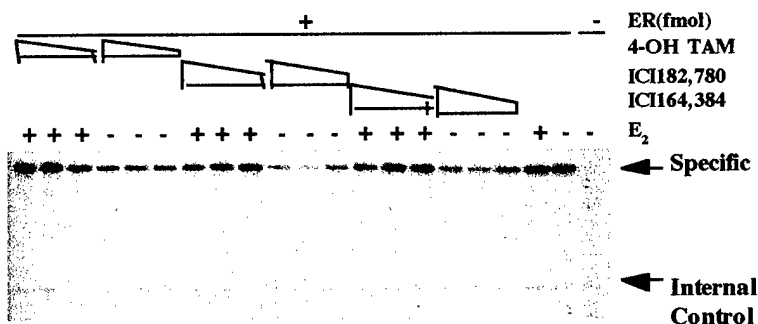


Figure 4. Antiestrogens inhibited ER-mediated transcription *in vitro*. The assay was carried out in the presence of vehicle, ICI 164,384 (4 to 400  $\mu$ M), ICI182,780 (4 to 400  $\mu$ M), 4-OH TAM (0.4 to 40  $\mu$ M),  $E_2$  (1  $\mu$ M) or combination of ICI164,384 and  $E_2$ , combination of ICI182,780 and  $E_2$  or combination of 4-OH TAM and  $E_2$ .

How do the antiestrogens inhibit ER-mediated transcription? We investigated the effects of the antiestrogens on ER DNA binding ability. ER-ERE complex migrated faster when ER bound to  $E_2$  than that in the absence of hormone or in the presence of 4-OH TAM (33). In ERE gel mobility shift assay (Fig. 5),  $E_2$  did not alter the migration rate of ER-ERE complex, further suggesting that ER already bound to  $E_2$ . ICI164,384 and ICI182,780 inhibited the formation of ER-ERE complex, which might contribute to the inhibitory effects of these two antagonists on ER-mediated transcription. 4-OH TAM does not affect the formation of ER-ERE complex. However, the migration of the ER-ERE complex in the gel was slightly slower when ER binds to 4-OH TAM as seen previously (33), suggesting that 4-OH TAM induced a conformation change of the complex which might affect the communication of ER and basal transcription factors and resulted in inhibition of ER-mediated transcription.

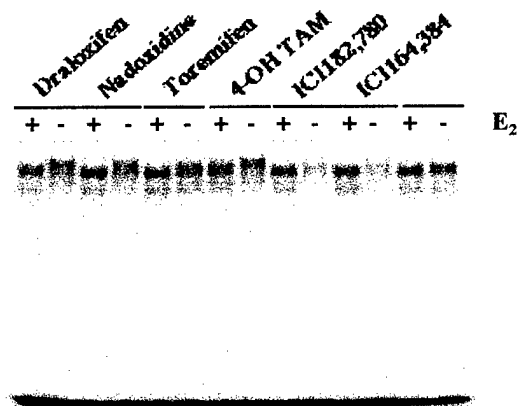


Figure 5. The effect of ligands on ER-ERE gel complex formation.

*SRC-1 Is Involved in ER-Mediated Transcription In Vitro*— SRC-1 is cloned as a steroid hormone receptor coactivator (18). It might be one of the communicators between ER and the basal transcription factors. To investigate the effect of SRC-1 on ER-mediated transcription *in vitro*, SRC-1 was constructed as a glutathione-*S*-transferase (GST) fusion protein (GST-SRC-1) and expressed in bacteria (see EXPERIMENTAL PROCEDURES). As shown in Figure 6, GST-SRC-1 slightly increased basal transcription level from the internal control template and the transcription from the specific template in the absence of ER. When added with ER, GST-SRC-1 significantly increased ER-mediated transcription in a concentration-dependent way.

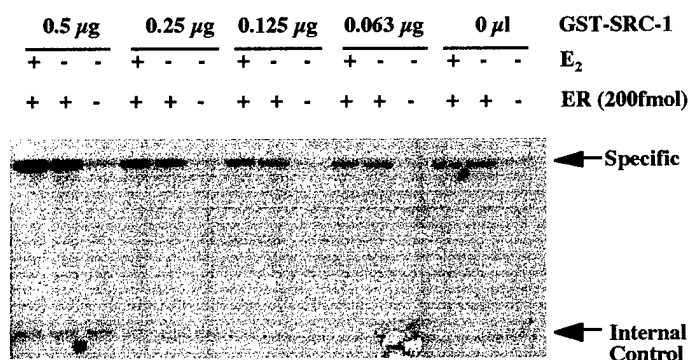


Figure 6. GST-SRC-1 significantly augmented ER-mediated transcription *in vitro*.

*The Proteins Which Associate with ER Hormone Binding Domain Are Required for the Full Transcription Activation Activity of the ER* — There are several proteins found to associate with ER hormone binding domain (HBD) in a hormone dependent manner (16-18, 26). These proteins bind to the ER hormone binding domain in the presence of  $E_2$  and its synthetic analog diethylstilbestrol (DES). They do not associate with ER hormone binding domain when antiestrogens such as 4-OH TAM, ICI164,384 and ICI182,780 are present.

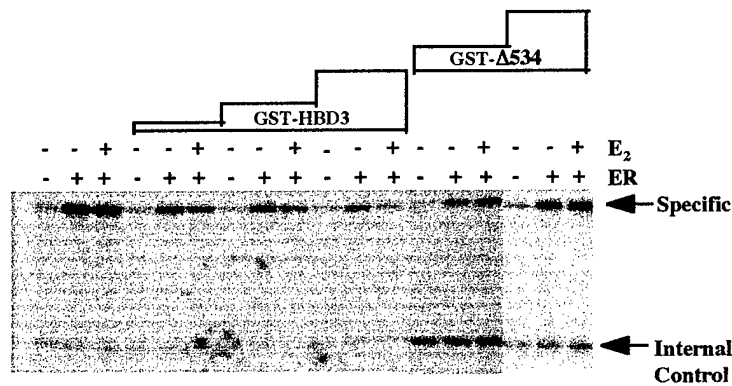


Figure 7. The ER HBD had dominant negative effect on ER-mediated transcription.

To determine the effects of these ER HBD associated proteins on ER-mediated transcription *in vitro*, we used GST-HBD3 that GST fuses to ER HBD (16) as a dominant negative competitor for the ER. Figure 7 showed that GST-HBD3 did not affect the basal transcription level, however, it significantly inhibits ER-mediated transcription. In contrast, GST-Δ534 (16) was used as a negative control. GST-Δ534 lacks of 535-595 amino acids of ER. It bound to hormone, but did not bind to ER associated proteins (16). GST-Δ534 did not affect ER-mediated transcription as shown in Figure 7.

If ER associated proteins are absolutely required for ER-mediated transcription, HeLa nuclear extracts depleted of the ER associated proteins will not support ER-mediated transcription *in vitro*. To test this hypothesis, we prepared depleted HeLa nuclear extracts by incubating with GST-HBD3-sepharose (see Experimental Procedures).

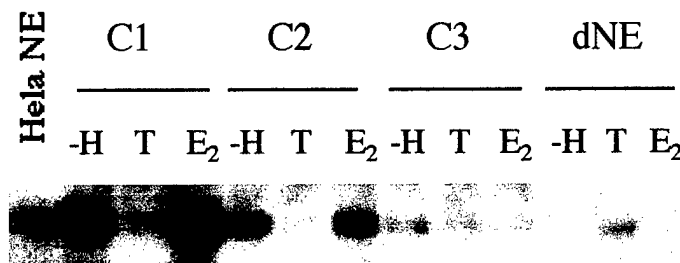


Figure 8. Far western blot. C1, C2 or C3 are the GST-HBD3-sepharose bound ER associated proteins after first, second or third depletion cycle. dNE is depleted nuclear extract.

Figure 8 shows that the ER associated proteins bound to GST-HBD3-sepharose and after three depletion cycle, an undetectable level of the associated proteins left in the depleted nuclear extract in the absence or presence of E<sub>2</sub> but quite high level in the depleted nuclear extract in the presence of 4-OHT. Surprisingly, all three depleted nuclear extract (no hormone, E<sub>2</sub> or 4-OHT) supported ER-mediated transcription *in vitro* to the same extent as the original nuclear extracts (Fig. 9).

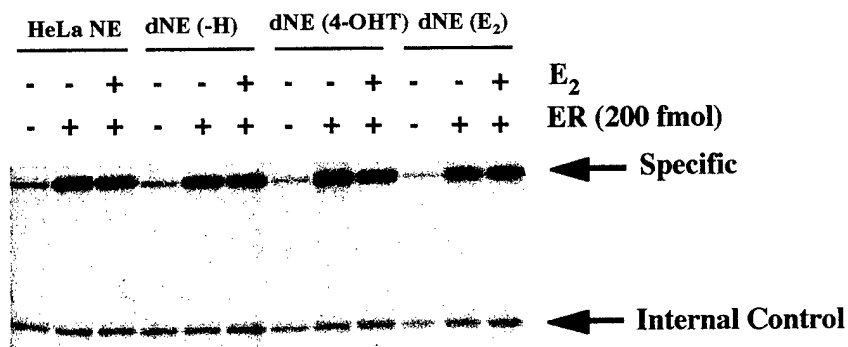


Figure 9. ER-mediated transcription *in vitro* in the depleted HeLa nuclear extracts (dNE). dNE (-H), dNE(E<sub>2</sub>) or dNE(4-OHT) were generated in the absence of hormones, estradiol or 4-hydroxytamoxifen, respectively.

### CONCLUSIONS

1. ER-mediated transcription *in vitro* is ligand-dependent; antiestrogens such as ICI164,384, ICI182,780 and 4-hydroxytamoxifen inhibited transactivation activity of the ER and estradiol induced ER-mediated transcription;
2. The hormone binding domain of the ER had dominant negative effect on ER-mediated transcription;
3. SRC-1 significantly augmented ER-mediated transcription *in vitro*.
4. Depletion of ER associated proteins from HeLa nuclear extracts did not affect ER-mediated transcription in this study.

## REFERENCES

1. Green, S. and Chambon, P. Nuclear receptors enhance our understanding of transcription regulation, *Trends. Gene.* 4: 309-314, 1988.
2. Evans, R. M. The steroid and thyroid hormone superfamily, *Science.* 240: 889-895, 1988.
3. Beato, M. Gene regulation by steroid hormones, *Cell.* 56: 325-44, 1989.
4. Tsai, M. J. and O'Malley, B. W. Molecular mechanisms of action of steroid/thyroid receptor superfamily members, *Annu. Rev. Biochem.* 63: 451-86, 1994.
5. Toft, D. O. and Gorski, J. A receptor molecule for estrogens: Isolation from the rat uterus and preliminary characterization, *Proc. Natl. Acad. Sci. USA.* 57: 1574-1581, 1966.
6. Jensen, E. V., Sujuki, T., Kawashima, T., Stumpt, W. E., Jungblut, P. W., and Desombre, E. R. A two-step mechanism for the interaction of estrodial with rat uterus, *Proc. Natl. Acad. Sci. USA.* 59: 632-638, 1968.
7. Green, S., Walter, P., Kumar, V., Brust, A., Bornert, J.-M., Argos, P., and Chambon, P. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A, *Nature.* 320: 134-139, 1986.
8. Greene, G., Gilna, P., Waterfield, M., Baker, A., Hort, Y., and Shine, J. Sequence and expression of human estrogen receptor complementary DNA, *Science.* 231: 1150-1154, 1986.
9. Pichon, M. F. and Milgrom, E. Clinical significance of the estrogen regulated pS<sub>2</sub> protein in mammary tumors, *Crit. Rev. Oncol. Hematol.* 15: 13-21, 1993.
10. Cavailles, V., Augereau, P., and Rochefort, H. Cathepsin D gene is controlled by a mixed promoter, and estrogens stimulate only TATA-dependent transcription in breast cancer cells, *Proceedings of the National Academy of Sciences of the United States of America.* 90: 203-7, 1993.
11. Krishnan, V., Porter, W., Santostefano, M., Wang, X., and Safe, S. Molecular mechanism of inhibition of estrogen-induced cathepsin D gene expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in MCF-7 cells, *Molecular & Cellular Biology.* 15: 6710-9, 1995.
12. Nardulli, A. M., Romine, L. E., Carpo, C., Greene, G. L., and Rainish, B. Estrogen receptor affinity and location of consensus and imperfect estrogen response elements influence transcription activation of simplified promoters, *Molecular Endocrinology.* 10: 694-704, 1996.
13. Garcia, M., Platet, N., Liaudet, E., Laurent, V., Derocq, D., Brouillet, J. P., and Rochefort, H. Biological and clinical significance of cathepsin D in breast cancer metastasis. [Review] [63 refs], *Stem Cells.* 14: 642-50, 1996.
14. Rochefort, H. Oestrogen- and anti-oestrogen-regulated genes in human breast cancer. [Review] [40 refs], *Ciba Foundation Symposium.* 191: 254-265, 1995.
15. Elliston, J. F., Fawell, S. E., Klein-Hitpass, L., Tsai, S. Y., Tsai, M.-J., Parker, M. G., and O'Malley, B. W. Mechanism of estrogen receptor-dependent transcription in a cell-free system, *Mol Cell Biol.* 10: 6607-6612, 1990.
16. Halachmi, S., Marden, E., Martin, G., MacKay, H., Abbondanza, C., and Brown, M. Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription, *Science.* 264: 1455-8, 1994.
17. Cavailles, V., Dauvois, S., Danielian, P. S., and Parker, M. G. Interaction of proteins with transcriptionally active estrogen receptors, *Proceedings of the National Academy of Sciences of the United States of America.* 91: 10009-13, 1994.
18. Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. Sequence and characterization of a

coactivator for the steroid hormone receptor superfamily, *Science*. 270: 1354-7, 1995.

19. Hong, H., Kohli, K., Trivedi, A., Johnson, D. L., and Stallcup, M. R. GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors, *Proc. Natl. Acad. Sci. USA*. 93: 4948-52, 1996.

20. Voegel, J. J., Heine, M. J., Zechel, C., Chambon, P., and Gronemeyer, H. TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors, *EMBO Journal*. 15: 3667-75, 1996.

21. Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X.-Y., Sauter, G., Kallioniemi, O.-P., Trent, J. M., and Meltzer, P. S. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer, *Science*. 277: 965-968, 1997.

22. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Makatain, Y., and Evans, R. M. Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300, *Cell*. 90: 569-80, 1997.

23. Hong, H., Kohli, K., Garabedian, M. J., and Stallcup, M. R. GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid and vitamin D receptors, *Mol. Cell. Biol*. 17: 2735-44, 1997.

24. Walfish, P. G., Yoganathan, T., Yang, Y., Hong, H., Butt, T. R., and Stallcup, M. R. Yeast hormone response element assays detect and characterize GRIP1 coactivator-dependent activation of transcription by thyroid and retinoid nuclear receptors, *Proc. Natl. Acad. Sci. USA*. 94: 3697-702, 1997.

25. Chakravarti, D., LaMorte, V. J., Nelson, M. C., Nakajima, T., Schulman, I. G., Juguilon, H., Montminy, M., and Evans, R. M. Role of CBP/P300 in nuclear receptor signalling [see comments], *Nature*. 383: 99-103, 1996.

26. Hanstein, B., Eckner, R., DiRenzo, J., Halachmi, S., Liu, H., Searcy, B., Kurokawa, R., and Brown, M. p300 is a component of an estrogen receptor coactivator complex, *Proceedings of the National Academy of Sciences of the United States of America*. 93: 11540-5, 1996.

27. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors, *Cell*. 85: 403-14, 1996.

28. Yao, T. P., Ku, G., Zhou, N., Scully, R., and Livingston, D. M. The nuclear hormone receptor coactivator SRC-1 is a specific target of p300, *Proceedings of the National Academy of Sciences of the United States of America*. 93: 10626-31, 1996.

29. Chiba, H., Muramatsu, M., Nomoto, A., and Kato, H. Two human homologues of *Saccharomyces cerevisiae* SWI2/SNF2 and *Drosophila* brahma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor, *Nucleic Acids Research*. 22: 1815-20, 1994.

30. Le Douarin, B., Zechel, C., Garnier, J., Lutz, Y., Tora, L., Pierrat, B., Heery, D., Gronemeyer, H., Chambon, P., and Losson, R. The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18, *EMBO J*. 14: 2020-33, 1995.

31. Lee, J. W., Ryan, F., Swaffield, J. C., Johnston, S. A., and Moore, D. D. Interaction of thyroid-hormone receptor with a conserved transcriptional mediator, *science*. 374: 91-4, 1995.

32. Nigro, V., Molinari, M., Armetta, I., de Falco, A., Abbondanza, C., Medici, N., and Alfredo Puca, G. Purified estrogen receptor enhances in vitro transcription, *Biochem. Biophys. Res. Commun*. 186: 803-810, 1992.

33. Brown, M. and Sharp, P. A. Human estrogen receptor forms multiple protein-DNA complexes, *J. Biol. Chem.* 265: 11238-43, 1990.
34. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. Accurate transcription initiations by RNA polymerase II in a soluble extract from isolated mammalian nuclei, *Nucl. Acids Res.* 11: 1475-89, 1983.
35. Beekman, J. M., Allan, G. F., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain, *Molecular Endocrinology.* 7: 1266-74, 1993.
36. Jordan, V. C. and Murphy, L. C. Endocrine pharmacology of antiestrogens as antitumor agents, *Endocrinol. Rev.* 11(4): 578-610, 1990.
37. Wakeling, A. E. Are breast tumours resistant to tamoxifen also resistant to pure antioestrogens?, *J Steroid Biochem Mol Biol.* 47: 107-14, 1993.